AWARD NUMBER: W81XWH-15-2-0029

TITLE: Enhancing the Immunogenicity of a Tetravalent Dengue DNA Vaccine

PRINCIPAL INVESTIGATOR: Maya Williams

CONTRACTING ORGANIZATION: Henry M. Jackson Foundation For the Advance of Military Medicine, Inc.
Rockville, MD 20852

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Dengue fever ranks among the top infectious diseases that afflict US Military personnel deployed overseas. Developing a successful vaccine to prevent dengue fever in DoD personnel is priority research area for the US DoD. Phase 1 clinical trials demonstrated that the Naval Medical Research Center's DNA based dengue vaccine is safe and well tolerated, but does not elicit a sufficient immune response. The objectives of this project are to conduct studies in non-human primates to enhance the immunogenicity of the vaccine by (a) testing different modes of delivery of dengue DNA vaccine for optimal humoral and T cell responses, and (b) testing the optimal delivery method in conjunction with other vaccine platforms using a heterologous prime-boost regimen. All necessary contracts are in place, the vaccine product is ready and the first vaccinations to address objective (a) are scheduled for early October 2016.

15. SUBJECT TERMS
Nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	14	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION:

Dengue fever ranks among the top infectious diseases that afflict US Military personnel deployed overseas. Developing a successful vaccine to prevent dengue fever in DoD personnel is priority research area for the US DoD. Phase 1 clinical trials demonstrated that the Naval Medical Research Center's DNA based dengue vaccine is safe and well tolerated, but does not elicit a sufficient immune response. The objectives of this project are to conduct studies in non-human primates to enhance the immunogenicity of the vaccine by (a) testing different modes of delivery of dengue DNA vaccines for optimal humoral and T cell responses, and (b) testing the optimal delivery method in conjunction with other vaccine platforms using a heterologous prime-boost regimen.

1. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Dengue, DNA vaccine, immunogenicity, non-human primate, vaccine delivery method

2. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Specific Aim 1: Select the vaccine delivery method which enhances the immunogenicity of the tetravalent dengue DNA vaccine. Vaccine delivery method plays an important role in the vaccine's performance. This is especially true for DNA based vaccines, where immunogenicity depends on the uptake of DNA by cells and de novo synthesis of antigens. The traditional method of delivery by intramuscular injection has proved sub-optimal for DNA vaccines, including TVDV. Technological development now makes it possible to consider delivery of DNA vaccines by needle-free devices intramuscularly or intradermally, and by electroporation. In this specific aim, we will evaluate the alternate methods of (a) intradermal electroporation, (b) intramuscular electroporation, and (c) intradermal liquid jet injection for enhancing TVDV in non-human primates.

Specific Aim 2: Develop an improved dengue vaccine using a heterologous prime boost approach. Heterologous prime-boost, in which two vaccines based on different platforms but using the same antigen has gained in popularity in recent years because of significantly enhanced immune responses. In this specific aim, we will evaluate this approach for enhancing TVDV. Specifically, we intend to test vaccine regimens that combine the following components: (1) TVDV administered using the vaccine delivery system selected from specific aim 1, (2) Live attenuated dengue virus vaccine (LAV), and (3) inactivated dengue virus vaccine.

What was accomplished under these goals?

Aim 1, Major task 1, Subtask 1: A contract with Ichor for use of their intramuscular and intradermal electroporation devices has been established.
Aim 1, Major task 1, Subtask 2: Due to the purchase of Bioject by another company, the Bioject devices were not available. In order to still meet aim 1, we identified and established a contract with another jet injection device company, PharmaJet.
Aim 1, Major Task 3, Subtask 1: Approval from the Wake Forest IACUC and USAMRMC has been received.
Aim 1, Major Task 3, Subtask 2: Non-human primates were screened for previous flavivirus exposure. Appropriate NHPs were identified, have been procured and are currently at Wake Forest. Vaccinations will proceed once the animals are released from quarantine.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

Ichor and PharmaJet will provide training on the use of their devices to personnel conducting the immunizations. Non-human primates will receive their first vaccinations in early October 2016 with subsequent vaccinations and blood draws for humoral and cellular immunogenicity measurements according to study protocols.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Due to the purchase of Bioject by another company, the Bioject devices were not available. In order to still meet aim 1, we identified and established a contract with another jet injection device company, PharmaJet.
PharmaJet offers equivalent intramuscular and intradermal devices with the exception of the maximum volume that can be delivered in one immunization with the intradermal device. We have increased the total number of injections such that the same dose of vaccine can be delivered.

Actual or anticipated problems or delays and actions or plans to resolve them

See above.

Changes that had a significant impact on expenditures

Delays in establishing contracts and the required change from Bioject to PharmaJet delayed expenditures. All required contracts have now been established.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals.

N/A

Significant changes in use of biohazards and/or select agents

N/A

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications.

  Nothing to report.

  Books or other non-periodical, one-time publications.

  Nothing to report.

Other publications, conference papers, and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	LCDR Maya Williams
Project Role:	PI
Nearest person month worked:	2
Contribution to Project:	Dr. Williams lead coordination/discussion with Wake Forest, PharmaJet and Ichor.
Funding Support:	Naval Medical Research Center
Name:	Dr. Peifang Sun - no change
Name:	Dr. Kanakatte Raviprakash
Project Role:	AI
Nearest person month worked:	2
Contribution to Project:	Dr. Raviprakash contributed to discussions with Wake Forest, Pharmajet and Ichor.
Funding Support:	Naval Medical Research Center
Name:	Mr. Dan Ewing - no change
Name:	Ms. Nish Nagabhushana - no change
Name:	Ms. Shyuer-Min Wang
Project Role:	Program manager
Nearest person month worked:	1
Contribution to Project:	Ms. Wang provided administrative support.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

See appendix, updated information is in red.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS:

Enhancing the Immunogenicity of a Tetravalent Dengue DNA Vaccine

JW140050 Joint Warfighter Medical Research Program
W81XWH-15-2-0029

PI: Maya Williams **Org:** Henry M. Jackson Foundation

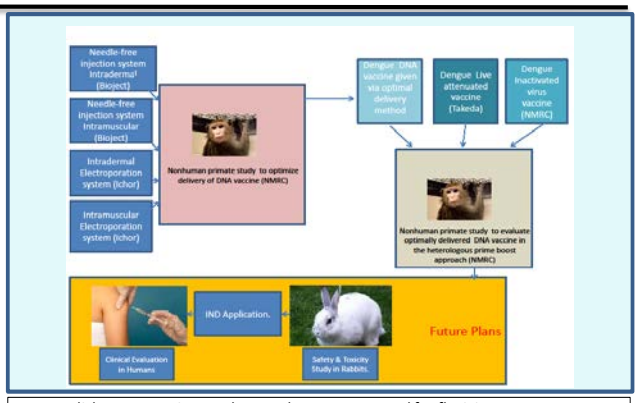
Study/Product Aim(s)

• Aim 1: Select the vaccine delivery method which enhances the immunogenicity of the tetravalent dengue DNA vaccine.

•Aim 2: Develop an improved dengue vaccine using a heterologous prime boost approach.

Approach

- Aim1: Conduct a nonhuman primate (NHP) study to determine the optimal delivery method for a tetravalent DNA dengue vaccine (TVDV). Compare immune responses elicited by TVDV administered with: (1) needle-free liquid jet injection (intradermal & intramuscular), (2) in vivo electroporation (intradermal and intramuscular).
- Aim2: Conduct a NHP study to evaluate immune responses induced by TVDV given by the best method identified in Aim 1 in a heterologous prime-boost vaccine approach using live attenuated and inactivated dengue virus vaccines.



Accomplishment: Vaccine product ready. NHPs screened for flavivirus exposure. Appropriate NHPs have been procured and first immunization date of early October 2016 has been established.

Timeline and Cost

Activities CY	15	16	17	18
Establish contracts, DNA vaccine prep, IACUC approval				
Aim 1 NHP study				
Aim 2 NHP study				
Final data analysis and reporting				
Estimated Budget ($K)	**$0.4K**	**$500K**	**$1000K**	**$399K**

Updated: (July 31, 2016)

Goals/Milestones

CY15 Goal

☑ DNA vaccine preparations

CY16 Goals

☑ Obtain IACUC approval for Aim 1 NHP study

☐Initiate NHP study for Aim 1

CY17 Goal

☐ Complete NHP study for Aim 1

☐ Initiate NHP study for Aim 2

CY18 Goal

☐ Complete NHP study for Aim 2

☐ Complete final data analysis

☐ **Comments/Challenges/Issues/Concerns**

- Delayed as purchase of Bioject by another company led to unavailability of their device, a new device has been identified and contract for its use has been established.

Budget Expenditure to Date

Projected Expenditure:

Actual Expenditure: $1340.42 Commitment: $528,586.07

9. APPENDICES

Appendix: Updated Support

APPENDIX

UPDATED KEY PERSONNEL CURRENT AND PENDING SUPPORT

LCDR MAYA WILLIAMS RESEARCH SUPPORT
CURRENT

Title: NMRC/WRAIR Global sequence analysis and antigenic characterization of currently circulating influenza strains using antigenic cartography
Time Commitments: 5% 0.6 calendar months
Supporting Agency: GEIS
NMRC Contact: Cheryl Carr, 301-319-7334
Performance Period: 10/1/15-09/30/16
Funding: $109,000
Goals and Specific Aims: Support GEIS partners by using sequence and phenotypic data in order to provide a comprehensive genetic and antigenic characterization of influenza viruses circulating around the globe. This information can be used to identify new and emerging strains of influenza as well as inform decision makers in the process to select the next season's influenza vaccine.
There is no overlap with the proposed project.

Title: Serological survey for Zika virus and other vector-borne pathogen exposures among DoD personnel deployed to Liberia for Operation United Assistance (OUA)
Time Commitments: 5% 0.6 calendar months
Supporting Agency: GEIS
NMRC Contact: Cheryl Carr, 301-319-7334
Performance Period: 10/1/15-09/30/16
Funding: $105,100
Goals and Specific Aims: This study will provide a more accurate picture of the efficacy of force health protection measures in place during OUA and will inform force health protection measures during future deployments to the region. Aim1: Determine the seroprevalence and seroincidence of antibodies against a range of vector-borne, zoonotic, and other pathogens among DoD personnel deployed to Liberia for Operation United Assistance (OUA) and stationed in other locations with potentially high burden for vector-borne diseases. Aim 2: Determine the pathogens associated with overt febrile illness.
There is no overlap with the proposed project.

Title: Investigation of the role of dengue non-structural proteins NS3 and NS5 in immune protection in a mouse model
Time Commitments: 5% 0.6 calendar months
Supporting Agency: Military Infectious Disease Research Program
NMRC Contact: Cheryl Carr, 301-319-7334
Performance Period: 10/01/15-09/30/16
Funding: $203,000
Goals and Specific Aims: This proposal will investigate the contribution of NS5 in addition to NS3 for enhancing neutralizing antibody titers and T-cell responses when co-immunized with a PIV vaccine that lacks these components. This proposal will also evaluate the

contribution of NS3 and NS5 alone to protect mice against DENV infection, and its underlying mechanism.
There is no overlap with the proposed project.

Title: The Role of Antibody-dependent Cell Cytotoxicity (ADCC) and Antibody-Dependent Cell-mediated Viral Inhibition (ADCVI) in Host Defense against Dengue Infection
Time Commitments: 5% 0.6 calendar months
Supporting Agency: Military Infectious Disease Research Program
NMRC Contact: Cheryl Carr, 301-319-7334
Performance Period: 10/01/15-09/30/16
Funding: $259,000
Goals and Specific Aims: The goal of this research is to investigate ADCC and ADCVI in natural dengue virus infections and vaccine trials. ADCC and ADCVI are areas which have been relatively under-looked compared to antibody neutralization and antibody-dependent enhancement; therefore this work will open a new window for seeking potential surrogate markers of immune protection to aid dengue vaccine development.
There is no overlap with the proposed project.

Title: A humanized mouse model for studying human immunology and pathogenesis of dengue virus infection
Time Commitments: 5% 0.6 calendar months
Supporting Agency: Military Infectious Disease Research Program
NMRC Contact: Cheryl Carr, 301-319-7334
Performance Period: 10/01/15-09/30/16
Funding: $151,000
Goals and Specific Aims: This proposal received funding to support preliminary experiments to explore if the DRAG-A mouse model is susceptible to infection by dengue virus. The current proposal is an extension of this work and would support a full characterization, validation and refinement of the model including additional metrics of the immune response.
There is no overlap with the proposed project.

Title: Anti-dengue human polyclonal antibodies from transchromosomic cattle as therapeutic and/or prophylactic agents against dengue virus infection
Time Commitments: 5% 0.6 calendar months
Supporting Agency: Military Infectious Disease Research Program
NMRC Contact: Cheryl Carr, 301-319-7334
Performance Period: 10/01/15-09/30/16
Funding: $185,000
Goals and Specific Aims: The hypothesis to be tested in this proposal is that fully human anti-dengue polyclonal antibodies produced in transchromosomic bovines are able to protect mice and non-human primates from dengue virus challenge.
There is no overlap with the proposed project.

Title: Use of the Dual Platform Immunization (DuPI) Approach to Develop a Tetravalent Dengue Vaccine **Time Commitments:** 8% 0.96 calendar months
Supporting Agency: DoD Peer Reviewed Medical Research Program, USAMRAA

NMRC Contact: Cheryl Carr, 301-319-7334
Performance Period: 10/1/14-09/30/16
Funding: $811,398/2years
Goals and Specific Aims: The goal of this project is to evaluate an immunization strategy that uses two different types of dengue vaccines administered simultaneously.
There is no overlap with the proposed project.

PENDING
Title: Development and Validation of the DRAG Humanized Mouse Model for Dengue Virus Infection and Vaccine Evaluation (JW160011)
Time Commitments: 15% 1.8 calendar months
Supporting Agency: the Congressionally Directed Medical Research Programs (CDMRP)
HJF Contact: Lisa Straker, 240-694-4016
Performance Period Proposed: 10/1/16-09/30/19
Funding: $2,666,773
Goals and Specific Aims: (#1) Determine if in vivo infection with a variety of serotypes and strains of DENV in humanized DRAGA mice results in infection associated with viremia, manifestations of disease, and the generation of a humanized immune response, and perform a comparative analyses of performance with a live dengue virus human challenge study. (#2) Characterize the immune response following in vivo vaccination of DRAGA mice with several dengue candidate vaccine formulations, determine efficacy by challenge with each dengue virus serotype, and compare the results with the live virus human challenge model.

DR. KANAKATTE RAVIPRAKASH RESEARCH SUPPORT
CURRENT
Title: Sustainment of Core Capability within DoD for Influenza serology and diagnostics to enable and support sero-epidemiological, vaccine efficacy, and related studies by GEIS partners
Time Commitments: 10% 1.2 calendar months
Supporting Agency: GEIS
NMRC Contact: Cheryl Carr, 301-319-7334
Performance Period: 10/01/15-09/30/16
Funding: $205K
Goals and Specific Aims: Goal of this project is to maintain NMRC as a DoD reference laboratory for influenza serology by developing assays and standard reagents for partnering DoD/other laboratories.

Title: Immune responses to sequential annual influenza vaccination in US Military personnel
Time Commitments: 10% 1.2 calendar months
Supporting Agency: GEIS
NMRC Contact: Cheryl Carr, 301-319-7334
Performance Period: 10/01/15-09/30/16
Funding: $223K
Goals and Specific Aims: Goal of this project is to determine if repeated annual vaccination against influenza results in stunted immune responses to vaccination. Study will provide data that could help in developing future vaccination recommendations.

Title: Anti-dengue Tc-bovine antibody
Time Commitments: 10% 1.2 calendar months
Supporting Agency: Military Infectious Disease Research Program
NMRC Contact: Cheryl Carr, 301-319-7334
Performance Period: 10/01/15-09/30/16
Funding: $259,039
Goals and Specific Aims: Goal of this project is to demonstrate efficacy of Tc-bovine derived anti-dengue human IgG in a non-human primate model.

Title: Convalescent plasma therapy to treat patients with severe influenza.
Time Commitments: 5% 0.6 calendar months
Supporting Agency: IDCRP (DoD Infectious Diseases Clinical Research Program)
IDCRP Contact: Samuel Davis, 301-816-8415
Performance Period: 08/01/16-04/30/17
Funding: $176K
Goals and Specific Aims: Goal of this project is to screen serum/plasma samples from human donors for anti-influenza antibody. The screen will help select plasma units for use in the clinical trial. Selected plasma units are also used in the preparation of IVIG to be used in the clinical trial.

PENDING
None